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DIETARY SUPPLEMENT WITH MARSH CINQUEFOIL, BURDOCK ROOT, FISH CARTILAGINOUS TISSUES, AND CEPHALOPODS

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ABSTRACT

With dietary supplements being important in the prevention and treatment of common diseases, Russian researchers focus on using local plants and aquatic animals. A new dietary supplement containing marsh cinquefoil extract, burdock root extract, fish cartilaginous tissues, and cephalopods was developed. The present study examines the potency of the ingredients, formulation, the qualitative and quantitative composition of the dietary product, the manufacturing process, as well ands the laboratory testing (organoleptic, physico-chemical, microbiological, toxicological) of the finished product. The shelf life of the product is 2 years when stored at 4-12°C in a dark place.

The recipe and technology of the developed additive were tested under production conditions at the enterprises of NPO Biolit (Tomsk). The functional properties of the supplement are confirmed by the results of clinical studies for rheumatoid arthritis. Prescription leads to a halving of the severity of clinical and laboratory symptoms in 80% of patients. A decrease in pain and the absence of internal stiffness were established, and positive dynamics in assessing the quality of life were established: improved mood, decreased frequency of headaches.

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Introduction

The development of dietary supplements that can be applied in the prevention and treatment of common diseases has been one of the priority areas for many researchers engaged in nutrition and medical sciences [1-3]. This has been particularly true for supplements produced with local plants and aquatic animals, as they are nutrient-rich [4-8]. We, therefore, developed and tested a new dietary supplement containing marsh cinquefoil extract, burdock root extract, fish cartilaginous tissues, and cephalopods. The dietary supplement is intended to be used in prevention and combination therapy of musculoskeletal conditions.

Materials and Methods

The materials used are natural raw materials of plant and animal origin, semi-finished products, and laboratory and experimental samples of the food supplement. Standardized and modified test methods have been used to assess the quality characteristics of the product.

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The pharmacological and biochemical characteristics of the natural active substances have been analyzed. This thorough analysis made it possible to scientifically substantiate the quantitative and qualitative composition of the product under development.

Marsh cinquefoil. Although all parts of the plant are used for medicinal purposes, roots are the most valued and have been registered in the Russian Federation as a medicine since 2008. The plant contains comaruman, which is pectin of cinquefoil Comarum palustre, as well as monoterpenes, namely pinene, terpineol, citronellal. The plant is a source of phenolic compounds (kaempferol, quercetin, apigenin, luteolin), catechins (epigallocatechin, gallocatechin), tannins, phenolic acids (chlorogenic, cinnamic, caffeic, p-coumaric, ferulic, salicylic), coumarins, umbelliferone, saponins, and sterols.

Water and water-ethanol extracts are produced from above-ground parts of the plant (flowers, grass) and underground parts (roots), which exhibit stress-protective and antitumor activity. Moreover, the antimicrobial and anti-inflammatory effects of the plant have been proven. The anti-inflammatory effect is an attribute of comaruman. The plant can be used for wound-healing purposes [9]. The leaves are used as a substitute for tea. Extracts are also used in beauty treatment. Water extract demonstrates an anticoagulant effect, while the water-ethanol extract exhibits a coagulant effect.

The quality of raw materials (grass) is subject to the following requirements: polyphenolic compounds=>1%; tannins in terms of tannin=>3%; catechins=>0.1%; sums of extractives=>15%, quercetin=>8.5%; kaempferol=> 6.1%, humidity<=13%.

Burdock roots. Burdock roots are a source of inulin (up to 45%), protein (up to 12.5%), essential bardan oil (up to 0.17%), tannins, palmitic and stearic acids, sitosterol, and stigmasterol. Burdock is rich in copper, titanium, boron, manganese, strontium, zinc, tin, vanadium, and iron.

For therapeutic purposes, the Burdock root is used as a diuretic, diaphoretic, and metabolic stimulant [10, 11]. Burdock decoction is recommended as a remedy for gout and kidney stones. Infusions and decoctions are applied as a blood purifier in combination with therapy for gastritis, duodenal ulcers, and abscesses.

Burdock root extract detoxifies the blood and increases urine production. The research has found that Burdock root compounds assist in bile production, and promote glycogen synthesis in the liver.

The quality of raw materials (Burdock root) is subject to the following requirements: inulin=>20%; sums of extractives =>35%; humidity <=14%.

Artrotin is a dry, easy-to-digest, water-soluble hydrolyzate of the fish cartilaginous tissues and cephalopods. Artrotin is a source of chondroitin sulfate and hyaluronic acid. Cartilage tissue is known to contain collagen fibers which tend to bind moisture. Thanks to the presence of sufficient amount of bound water, cartilage tissue exhibits elasticity, which is necessary for proper joint functions. Therefore, regular consumption of chondroitin sulfate and hyaluronic acid is important for maintaining the necessary healthy articular cartilage, joint fluid, and as well as strong bone tissue [3].

Artrotin is prescribed along with non-steroidal anti-inflammatory drugs in combination therapy to treat joint diseases. The components of Artrotin are involved in building ligaments, tendons, nails, and skin. They also assist in regulating calcium deposits in bone tissue and help relieve joint pain. Artrotin is also applied to boost injury recovery, enhance exercise and athletic performance, strengthen hair and nails, and increase skin elasticity.

High-quality artrotin must comply with the following requirements: glucosamine=>0.5%; sulfate ions=>1% (mass fractions), humidity<=7%.

Esobel is an organically produced mud from highly mineralized Siberian lakes. Esobel contains mineral salts (cations Na+, Ca2+, Mg2+, K+, and anions Cl-, SO42-, CO32-, HCO3-) as well as organic compounds (fulvic and humic acids, amino acids, and prostaglandins).

Esobel exhibits anti-inflammatory activity by reducing pain and hyperemia and suppresses connective tissue proliferation. Moreover, Esobel assists in maintaining and restoring joint mobility.

The quality of Esobel is subject to the following requirements: calcium=>0.6%; carbonates=>0.55%; magnesium=>4.5%; sulfates=>6.7%; chlorides =>56%.

Results and Discussion

Taking into account the qualities of the above-mentioned ingredients, we determined the following qualitative and quantitative composition of the dietary supplement **(Table 1)**.

Table 1. Dietary supplement: Qualitative and quantitative composition

Components	**Content (kg)**
Marsh cinquefoil (grass)	133.0
Burdock roots (ground)	58.0
Total	**191.0**
Artrotin	50.0
Esobel	10.0
Marsh cinquefoil, dry extract	20.0
Burdock, dry extract	20.0
Total	**100.0**

The dietary supplement was manufactured in the consecutive stages presented in **Figure 1**.

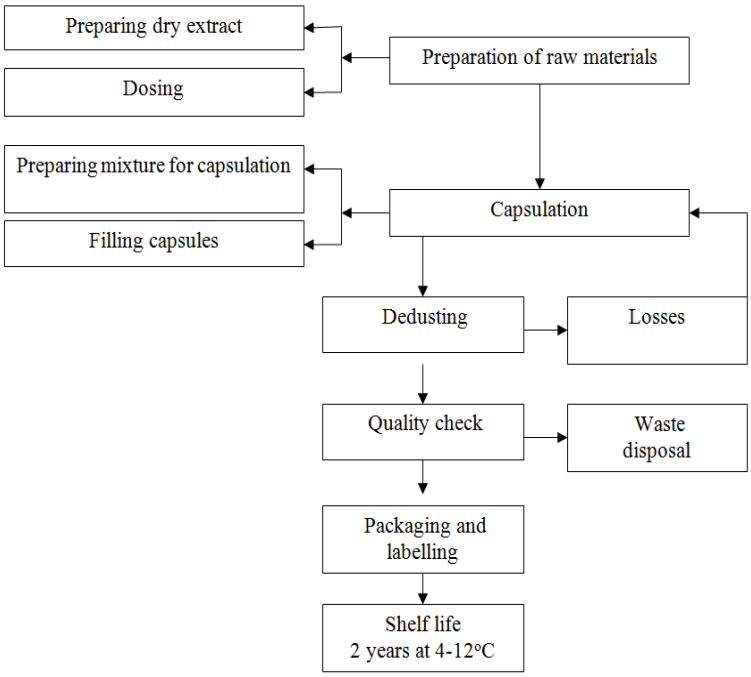

Figure 1. Dietary supplement: The manufacturing process

Raw materials preparation. To ensure the manufacturing process adheres to current legislation and industry regulations, raw materials were checked upon receipt with the following requirements met:

- The date of manufacture, the name of the raw material, producer's contacts were clear, correct, and complete on every package;
- The specifications on the incoming quality and safety control were confirmed by an accredited laboratory;
- The accompanying documents and the statement of production admission (a green strip on the label) were accurate.

Burdock and marsh cinquefoil dry extracts preparation process.
Ground burdock roots (diam. 2.0-3.0 mm) were loaded into a food boiler with water preheated to 60°C and left for 2 hours (with the anchor mixers switched on and the steam generator set at 80°C). The extraction process was repeated three times with an interval of 2 hours **(Table 2)**.

Table 2. Burdock roots extraction.

Components	Content (kg)
Ground burdock roots	58.0
Purified water	290.0
Liquid extract	180.0
Thick extract	90.0
Dry extract	20.0

The preparation of the dry extract from ground marsh cinquefoil (diam. 1.0-2.0 mm) was similar to the preparation of the dry extract from Burdock roots **(Table 3)**.

Table 3. Marsh cinquefoil extraction.

Components	Content (kg)
Ground marsh cinquefoil	133.0
Purified water	665.0
Liquid extract	430.0
Thick extract	210.0
Dry extract	20.0

The extraction process was controlled by monitoring the quality of the raw materials and production parameters (extraction temperature and extraction time).

The liquid extract was filtered using a milk filter and a nonwoven filter (60% polyester and 40% polypropylene) with 60 µm filter fineness. The extracts obtained were brown, slightly opalescent, with a mass fraction of 7-10%.

To obtain the thick extract (with a dry matter content of 60%), evaporation at 70-80°C was performed in a food boiler for 5 hours. Then, the thick extract was filtered through a sieve with a grid diameter of 2 mm and sent to freeze-dry (at -10-12°C), with residual moisture of 5%.

The next step in the manufacturing process included dosing (in strict accordance with the production guidelines), checking the quality of the ingredients, and mixing. The mixing process carried out in a mixer at a speed of 800 RPM lasted for 1 hour.

Once the mixture was ready for capsulation, a capsule machine was employed to enclose the mixture into hard gelatin capsules (0.51-0.61 g per capsule). Afterward, capsules were dedusted on sieves.

Samples of the finished product (100 g) underwent laboratory testing to assess quality and safety as stated in the production guidelines. **Tables 4 and 5** show the organoleptic, physico-chemical, microbiological, and toxicological characteristics of the dietary supplement.

Table 4. Organoleptic characteristics

Indicator	**Description**
Appearance	Powder in hard capsules
Color	Brown-grey with dark speckles
Taste	Salty
Smell	Similar to fresh seafood smell
Physico-chemical characteristics	
Indicators	**Normal value**
Tannins (tannin) mg/caps., not less than	15.0
Glucosamine, mg/caps., not less than	8.5
Chondroitin sulfate, mg/caps., not less than	12.5
Capsule dissolvent, min., no longer than	15.0
Moisture, no more than, %	5.0

Table 5. Dietary supplement: toxicological and microbiological characteristics

Indicator	**Acceptable**	**Factual**
Toxic elements, mg/kg, not more than		
Lead	10.0	0.10
Arsenic	12.0	2.9
Cadmium	0.2	0.021
Mercury	0.5	0.001
Pesticides, mg/kg, not more than		
HCH (α, β, γ-isomers)	0.2	less than 0.005
DDT and its metabolites	2.0	less than 0.005
Heptachlor	not allowed	none
Aldrin	not allowed	none
Microbiological characteristics		
CFU/g, not more than		
KMAFanM	$1 \cdot 10^4$	less than 10
product weight (g)		
BGKP (coliforms)	0.1	none
Pathogens (Salmonella included)	10	none
E. Coli	1.0	none
S. Aureus	1.0	none
CFU/g, not more than (for a dietary supplement with plants and seafood)		
Yeast and mould	200	less than 10

The dietary supplement was determined to have a two-year shelf-life period when stored at 4-12°C in a dark place.

The dietary supplement was manufactured and tested by Biolit (a scientific and production company located in Tomsk, Russia) in full compliance with ISO 9001, 22000, and GMP to ensure the quality and identity of the ingredients and guarantee the safety and potency of the finished product.

Conclusion

To evaluate the functional properties of the dietary supplement, a clinical trial was performed. Patients with rheumatoid arthritis, which is a chronic inflammatory disorder affecting joints, were administered the dietary supplement as part of the combination therapy. As a result, the severity of symptoms halved in 80% of the patients. Patients reported noticeable relief in joint pain and stiffness, improved quality of life with better mood, less frequent headaches, reduced muscle weakness, and increased work capacity.

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Conflict of interest: None

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Ethics statement: The study was conducted according to the guidelines of the Declaration of Helsinki.

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